Current Topics in Biochemical Research
Vol. 13, *2*, 2011

Mini-Review

The apelin-APJ and renin-angiotensin systems

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ABSTRACT

Apelin is a 36 amino-acid vasoactive peptide originally isolated from bovine stomach extracts as a selective endogenous ligand of orphan receptor, APJ, which was genetically identified to have closest identity to the angiotensin II type 1 receptor (AT-1). Previous studies have shown that both apelin and its receptor, APJ, distribute in a wide variety of tissues including central nervus system, heart, lungs, and kidneys, and apelin-APJ system has been found to have relevant roles in human physiology, such as the regulation of cardiovascular, gastrointestinal, and immune functions, bone physiology, fluid homeostasis, and embryonal development of cardiovascular system. Interestingly, in spite of high homology between APJ receptor and AT-1 receptor, as well as high similarity in the tissue expression of both receptors, previous reports showed opposing actions between apelin-APJ system and angiotensin II (Ang II)-AT-1 system in a number of physiologic and pathophysiologic settings. In this mini-review, we highlight the actions of these two systems, and discuss the physiologic and pathophysiologic significance of these systems.

KEYWORDS: apelin, APJ receptor, renin-angiotensin system

INTRODUCTION

Apelin was originally isolated from bovine stomach extracts, as a selective endogenous ligand for a G-protein coupled receptor, APJ, which was originally identified as a gene with closest identity to the angiotensin II type 1 receptor (AT-1) [1, 2]. Both apelin and APJ receptor were found to distribute in a wide variety of tissues including central nervus system (CNS), heart, lungs, and kidneys [3-7], and apelin-APJ system has been shown to have relevant roles in the regulation of cardiovascular, gastrointestinal, and immune functions, bone physiology, fluid homeostasis, and embryonal development of cardiovascular system [8-10]. In spite of high homology between APJ receptor and AT-1 receptor, as well as similar patterns of tissue expression for both receptors, previous reports showed opposing actions between apelin-APJ system and angiotensin II (Ang II)-AT-1 system in a number of physiologic and pathophysiologic settings [11-17]. Apelin mediates counter-regulatory actions to Ang II on cardio-vascular function [11-17]. Furthermore, recent reports showed anti-fibrotic actions of apelin in cardiovascular system that counteracts to Ang II [17, 18]. In this mini-review, we highlight the actions of these two systems, and discuss the physiologic and pathophysiologic significance of these two systems.

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Apelinergic peptides and APJ receptor

In 1993, O'Dowd *et al.* [1] identified a gene with closest identity to the angiotensin II type 1 receptor (AT-1). A detailed analysis revealed that this gene codifies a G-protein-coupled receptor with 380 aminoacids, and was named APJ. While the APJ receptor shows high homology with AT-1 receptor, i.e., 115 aminoacids (AA) (30%) of the total sequence and 86 AA (54%) in transmembrane regions [1],

and the APJ and AT-1 receptors also show high similarity in the tissue expressions [6], Ang II does not bind to APJ receptor [1, 2]. APJ receptor was kept orphan until 1998, when Tatemoto *et al.* [2] isolated a 36 AA peptide from bovine stomach extracts, which they named apelin. The studies regarding the tissue distribution of APJ receptor in rats, mice, and humans showed that it is expressed in a wide range of tissues, including lung, heart, kidney, adipose tissue, and throughout the brain [1, 3, 6, 7, 19, 20]. In the cardiovascular system, the APJ receptor was found in the endothelial cells of small intramyocardial, renal, pulmonary and adrenal vessels, in coronary arteries, in endocardial endothelial cells, and in vascular smooth muscle cells [7].

Apelin is synthesized as a 77 AA pre-propeptide, which is codified in a gene with 1,726 base pairs that include 3 exons located in Xq25-26.1 in humans, and is cleaved to a mature 36 AA peptide [2]. Previous studies identified different isoforms of apelin that are thought to exist *in vivo*. These include apelin-36 (apelin42-77), apelin-17 (apelin61-77), apelin-13 (apelin65-77), and apelin-13 in its pyroglutaminated form [(Pyr^1)apelin-13], i.e., with N-terminal glutamate residue [2]. Expression of apelin and APJ in the cardiovascular system suggests involvement of apelin-APJ system in cardiovascular function, and implies links to the renin-angiotensin system (RAS) pathway. In their cardiovascular action, shorter isoforms seem to be more powerful, and (Pyr^1)apelin-13 is the most potent [19] and abundant form in cardiac tissue [21]. In general, apelin-36 is thought to have limited biological activities. However, after proteolysis and post-translational changes, apelin-36 produces biologically more active peptides, predominantly (Pyr^1)apelin-13, in which pyroglutamination preserves biological function and prevents enzyme degradation [9].

In the adult, high level expression of APJ is found in the heart, lung, and kidney [1, 3, 6, 7, 19]. In human myocardium, the density of APJ receptors is similar to that of Ang II receptors, and though the RAS involves multiple receptors for both Ang II and other Ang I-derived peptides with different functional profiles, radioligand binding assays indicated that apelin binds heart tissue with kinetics suggesting a single binding site, and the various apelin peptides have shown similar basic functional profiles, despite dissimilar pharmacological properties. While pre-proapelin mRNA is abundantly found in the CNS and in the placenta, and in more moderate volume in kidneys, heart, lungs and mammary gland, apelin is biologically active in the myocardium, in cardiac endothelium, and in the endothelium of large vessels and small veins and arteries [8].

Physiological actions of the apelin-APJ and renin-angiotensin systems

The apelin-APJ pair is intrinsically related to the Ang II-AT-1 pair in regulating the pathophysiology of cardiovascular function. Apelin attenuates the vasoconstrictor effects of Ang II through both nitric oxide (NO)-dependent and -independent pathways [12, 13]. In addition, APJ-deficient mice showed an increased vasopressor response to Ang II [14]. Because the hemodynamic effect of apelin was abrogated in the presence of a nitric oxide synthase (NOS) inhibitor, it was suggested that apelin may lower blood pressure via a nitric oxide-dependent mechanism [14, 22]. Apelin also causes endothelium-dependent vasodilation through the phosphorylation of Akt and endothelial nitric oxide synthase (eNOS) that promotes NO release [22].

In heart failure, serum level of Ang II increases, whereas apelin level decreases [23, 24]. Apelin is a most potent endogenous inotropic agents, and apelin increases cardiac contractility in atrial strips [21] and whole rat hearts [25], and increases sacromere shortening in cardiomyocytes [26]. Apelin infusion also increases myocardial contractility *in vivo*, and chronic dosing of apelin causes a sustained increase in cardiac output without inducing left ventricular hypertrophy [27]. Iwanaga *et al.* [28] showed a functional counter-regulation of apelin and Ang II in a rat model of progressive heart failure. In their study, although apelin and APJ receptor mRNA showed no change in the compensatory left ventricular hypertrophy (LVH) stage, these were markedly down-regulated in the heart failure (HF) stage. The rats were treated with angiotensin type 1 receptor blocker (ARB), matrix metalloproteinase inhibitor, or β-beta blocker from the LVH stage, and although the functional improvements were similar among the three treated groups, restoration of cardiac apelin

and APJ expression was observed only in the ARB group. Furthermore, in Ang II-infused rats, cardiac apelin mRNA was decreased and its restoration was achieved by treatment with ARB. These results established a direct counter-regulatory influence of Ang II in apelin and APJ expression in the context of heart failure and suggest that the beneficial effects of RAS inhibition may at least in part be explained by restoration of apelin signalling. Furthermore, protective effects of apelin against cardiovascular fibrosis have also been shown more recently in a model of Ang II-induced cardiovascular fibrosis [18] and in those during the treatment with ARB [17].

In glucose metabolism, apelin acts to reduce plasma glucose concentration after a glucose load, through insulin-dependent and -independent pathways [29]. In apelin-deficient mice, diminished insulin sensitivity, increased serum insulin concentration, and decreased insulin-induced Akt phosphorylation in the muscle lysates were observed [30]. It was concluded that apelin is necessary for the maintenance of insulin sensitivity *in vivo*. In contrast, Ang II induces insulin resistance at a cellular level by increasing oxidative stress and altering insulin signalling [31].

Interactions of the apelin-APJ and renin-angiotensin systems

Recent reports also showed the direct interactions between apelin-APJ and RAS at both molecular and transcriptional levels. Chun *et al*. [15] demonstrated in rat primary aortic smooth muscle cells that apelin inhibits Ang II signalling pathways including extracellular signal-regulated kinase phosphorylation and activation of transcriptional targets, such as nuclear factor-κB. A recent report by Siddiquee *et al.* [18] also showed that apelin blocks Ang II activation of Rho kinase pathway in human aortic smooth muscle cells, which is associated with the induction of plasminogen activator inhibitor type-1 (PAI-1) gene expression by Ang II. Ang II also appears to counteract apelin-APJ system in the context of apelin and APJ receptor gene expressions. Infusion of Ang II in rats has been shown to reduce cardiac apelin mRNA expression, which was restored by the treatment with ARB [28].

Chun *et al.* [15] also showed that AT-1 and APJ receptors can form heterodimers. Although this heterodimerization has only been observed *in vitro* at high receptor densities and may not accurately reflect the *in vivo* situation in native tissues, this shows that AT-1 and APJ receptors can physically associate presumably on the cell membrane, and may influence downstream signalling in a stoichiometric fashion with each other. Furthermore, a recent report by Sun *et al.* [32] showed that, in human embryonic kidney 293 cells, coexpression with APJ significantly suppressed the phosphorylation of extracellular signal-regulated kinases 1/2 (ERK1/2) induced by Ang II-AT-1, whereas apelin, through activated APJ, abolished this attenuation independently of its heterodimerization. They concluded that non-activated APJ may suppress Ang II-AT-1 signalling, whereas this ligand-independent function was diminished with apelin activation.

On the other hand, angiotensin and apelin share signalling pathways. In relation to myocardial signalling, apelin, as well as Ang II-AT-1, exerts a positive inotropic effect through phospholipase C (PLC), protein kinase C (PKC), sodium-hydrogen exchanger (NHE) and sodium calcium exchange (NCX), resulting in a greater calcium entry and increased myofilament sensitivity, mediated by intracellular alkalinization [25]. Apelin and angiotensin signalling in smooth muscle also shares similarities. In smooth muscle cells, apelin, as well as Ang II, causes vascular smooth muscle contraction via phosphoinositide hydrolysis and a rise in intracellular calcium, which causes phosphorylation of the myosin light chain (MLC) [33]. Such conservation of signalling in muscle cells stands in stark contrast to the differing functional profiles of apelin and Ang II.

Apelin and Ang II also relates in the regulation of enzymatic processing. Ang II is created from angiotensin I (Ang I) through cleavage by the peptidyl dipeptidase angiotensin converting enzyme (ACE). Recently, carboxypeptidase angiotensin converting enzyme 2 (ACE2) has been identified as an important processing enzyme that may function to counterbalance ACE. ACE2 converts Ang II to a seven-amino acid form that is biologically active in cardiovascular physiology,

and may serve to counteract the harmful actions of Ang II [34]. ACE2 also cleaves Ang I, thus regulating Ang II production, and generating a nonapeptide that has biological activity. It is currently thought that ACE2 also plays the role of apelin degradation through highly efficacious cleavage of the terminal C phenylalanine from the apelinergic peptides [35]. Interestingly, ACE2 expression is primarily in the endothelial cells of the vasculature, where apelin and APJ are both preferentially expressed. Such correlation therefore suggests the high relevance of apelin in cardiovascular regulation in relation with RAS [36].

CONCLUSION

Previous studies showed reciprocal counter-regulation between the apelin-APJ and RAS. However, further studies regarding the cross-talk between these two pathways at the cellular level, including at the level of cell surface receptors, cytoplasmic signalling proteins, and enzymatic processing, are necessary to define the exact mechanism of counter-regulation. Furthermore, studies regarding the role of apelin-APJ system in the disease settings are considered important. These studies will provide insights into the role of Ang II inhibition, and more importantly, raises the possibility of therapeutic options targeting apelin-APJ as well as RAS in various pathological settings.

CONFLICT OF INTEREST STATEMENT

None

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